PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 30291PC01	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/DK 03/00504	International filing date *(day/month/year)* 18.07.2003	Priority date *(day/month/year)* 22.07.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant PLANTIC APS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
31.01.2004	14.10.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Helliot, B Telephone No. +49 89 2399-7793

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK 03/00504

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-32 as originally filed

Sequence listings part of the description, Pages

1-3 as originally filed

Claims, Numbers

1-31 filed with telefax on 20.09.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☒ the claims, Nos.: 32

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-31
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-31
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-31
	No:	Claims	

2. Citations and explanations

see separate sheet

ITEM V:

1- INTRODUCTION

The following documents (D1-D2) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: Revenkova E. et al. (1999), Involvement of *Arabidopsis thaliana* ribosomal protein S27 in mRNA degradation triggered by genotoxic stress. EMBO Journal 18(2): 490-499.

D2: WO-A-9630402

The present application relates to a method of identifying a nucleic acid sequence encoding a product that is involved2- in cell growth regulation in an eukaryotic target organism.

3- NOVELTY (Art. 33(2) PCT) and INVENTIVENESS (Art. 33(3) PCT)

3-1 No cited prior-art document discloses a method of identifying a nucleic acid sequence encoding a product that is involved in cell growth regulation in an eukaryotic target organism, the method comprising the step of:

a) providing a plant that is genetically modified to have tissue exhibiting, relative to the tissue of its non-genetically modified parent plant, accelerated growth,

b) subjecting a multiplicity of the genetically modified plant, or parts thereof to a mutagenisation treatment,

c) selecting from the thus treated plants or parts thereof mutant plants having, relative to the wild type plant or the genetically modified plants of step a), a phenotype characterized by an altered morphological structure or an altered colour,

d) identifying in said selected mutant plants nucleic acid sequence(s) having a nucleic acid sequence which is different from the corresponding sequence(s) in a non-mutagenised wild type plant or the genetically modified plant, and using said nucleic acid sequence(s),

e) identifying in the eukaryotic target organism a target nucleic acid

sequence comprising a sequence encoding a product that is involved in cell growth regulation.

Therefore, the subject-matter of independent **claim 1** is considered novel in the sense of Art. 33(2) PCT.

Moreover, the subject-matter of **claim 1** does involve an inventive step over the disclosure of D1 (Art. 33(3) PCT).

D1, which is considered as the closest prior art, describes an *Arabidopsis* mutant with elevated sensitivity to DNA damaging treatments wherein:

I) a collection of 800 families of *Arabidopsis* containing random insertions of *Agrobacterium tumefaciens* T-DNA is screened for individuals with elevated sensitivity to two kinds of DNA-damaging agents: UV-C and methyl methane sulfonate (MMS) (p. 491, c. 1, l. 38-41),

ii) one family is found to contain individuals with increased sensitivity to both UV-C and MMS (p. 491, c. 1, l. 50-51) on the basis of morphological observation (p. 491, c. 1, l. 52-53 and Fig. 1),

iii) the mutant locus is characterized and corresponds to an open reading frame encoding a protein of 86 amino acids and a database search reveals that the deduced amino acid sequences shares 77% identity and 83% similarity to the rat ribosomal protein S27 (p. 491, c. 2, l. 8-10 and l. 15-20),

iv) in contrast to the wild type, the growth of ars27A mutant , (ars27A is the homologue of S27 in *Arabidopsis*), is accompanied by characteristic developmental abnormalities, like tumor-like structures on the main root, while the wild-type seedling in the same conditions never displayed such a trait (p. 493, c. 2, l. 3-11).

The subject-matter of **claim 1** differs from that of D1 in that a population of genetically modified plants, which have been modified to obtain an increased cell division frequency leading to an accelerated growth and development, while typically retaining an overall morphological structure similar to the wild type plant from which they are derived, is created in order to find knocked-out or activated genes involved in the regulation of plant cell growth.

Thus, the technical problem to be solved by the subject-matter of said **claim 1** may be regarded as providing an alternative method to that of D1.

In view of the absence of any indications which would have lead the skilled person to apply the method of D1 to a population of genetically modified plants, which are modified to have an increased cell division frequency leading to an accelerated growth and development, it would not have been obvious for the skilled person to develop a method of identifying a nucleic acid sequence encoding a product that is involved in cell growth regulation in an eukaryotic target organism as disclosed in **claim 1.**

Therefore, the subject-matter of **claim 1** involves an inventive step (Art. 33(3) PCT).

3-2 Dependent **claims 2-30** further define specific embodiments of the novel and inventive method of claim 1.
Dependent **claims 2-30** are hence also considered to meet the requirements of Art. 33(2) and (3) PCT.

3-3 Moreover, no cited prior-art document discloses a method of determining the tumor suppressor activity, if any, of a gene product encoded by an eukaryotic cell gene, the method comprising the different step as defined in said claim 31 and wherein a gene involved in cell cycle regulation is identified first in a wild-type or genetically modified plant and then in an eukaryotic cell.

Therefore, the subject-matter of independent **claim 31** is considered novel in the sense of Art. 33(2) PCT.

Moreover, the subject-matter of **claim 31** does involve an inventive step over the disclosure of D2 (Art. 33(3) PCT).

D2 relates to a method of identifying a tumor suppressor gene comprising (a) identifying an overproliferation phenotype in a genetic mosaic; and (b) isolating a gene that is mutated in cells exhibiting said overproliferation phenotype, wherein:

I) the genetic mosaic is achieved by induction of somatic cells in an animal that is heterozygous for an induced mutation to become homozygous for the mutation, at any desired developmental stage,

ii) the mutation can be induced by any known method, e.g., X-ray exposure or chemical mutation exposure or insertion of a transposable element (p. 80, §. 1),

iii) the lats gene is identified in *Drosophila melanogaster* showing over

proliferation mutation (p. 83, l. 30-32)
iv) the lats homologue genes in mammalian is isolated and characterized in mouse (p. 99, l. 21 - p. 100, l. 16), in human (p. 100, l. 17 - p. 101, l. 20),
v) drosophila lats gene is cloned into an appropriate vector which contains the necessary elements for the transcription and translation (p. 19, l. 30-32),
vi) the plant expression vectors comprises the nopaline synthetase promoter region or the cauliflower mosaic virus 35S RNA promoter (p. 21, l. 4-7),
vii) chemical mutagenesis can be carried out to reduce or destroy endogenous lats function in order to increase the growth (p. 70, l. 28-29).

The subject-matter of **claim 31** differs from that of D2, which is considered as the closest prior art, in that the gene putatively involved in cell growth regulation is first identified in a wild-type or genetically modified plant, which is subjected to a mutagenisation treatment, before its identification in an eukaryotic cell and in that the mutated plant is subsequently reversed with the gene sequence identified in the eukaryotic cell, such reversion being indicative of tumor suppressor activity.

In view of the absence of any indications which would have lead the skilled person to I) identify tumor suppressor genes in a population of wild-type or genetically modified plants, which is subjected to a mutagenisation treatment, before its identification in an eukaryotic cell and then to ii) reverse the mutated plant population to its wild type phenotype, such reversion being indicative of tumor suppressor activity, it would not have been obvious for the skilled person to develop a method of determining the tumor suppressor activity, if any, of a gene product encoded by a eukaryotic cell gene as disclosed in **claim 31**.

Therefore, the subject-matter of **claim 31** involves an inventive step (Art. 33(3) PCT).

4- REMARK

It is drawn to the attention of the Applicant that **claim 27,** which relates to a method according to any of the preceding claims, wherein the effect of expressing the target nucleic acid sequence is assayed in an *in vivo* model, would be considered unallowable by some National/Regional Authorities, insofar as the eukaryotic target organism includes humans as disclosed in the present description (see p. 15, l. 15-20).

CLAIMS

1. A method of identifying in a eukaryotic target organism a nucleic acid sequence encoding a product that is involved in cell growth regulation in said target organism, the method comprising the steps of:

(a) providing a plant that is genetically modified to have tissue exhibiting, relative to the tissue of its non-genetically modified parent plant, accelerated growth,

(b) subjecting a multiplicity of the genetically modified plant, or parts thereof to a mutagenisation treatment,

(c) selecting from the thus treated plants or parts thereof mutant plants having, relative to the wild type plant or the genetically modified plant of step (a), a phenotype characterised by an altered morphological structure or an altered colour,

(d) identifying in said selected mutant plants nucleic acid sequence(s) having a nucleic acid sequence which is different from the corresponding sequence(s) in a non-mutagenised wild type plant or the genetically modified plant, and, using said nucleic acid sequence(s),

(e) identifying in the eukaryotic target organism a target nucleic acid sequence comprising a sequence encoding a product that is involved in cell growth regulation.

2. A method according to claim 1, wherein the wild type plant or the parent plant for the genetically modified plant of step (a) is selected from a group consisting of *Lotus japonicus*, *Medicago truncatula*, *Oryza sativa*, *Antirrhinum majus* and *Arabidopsis thaliana*.

3. A method according to claim 2, wherein the wild type plant or the parent plant is *Arabidopsis thaliana*.

4. A method according to any of claims 1-3, wherein the accelerated growth of tissue of the genetically modified plant is due to overexpression of a gene selected from the group consisting of a gene coding for a cyclin, a gene coding for a transcription factor including E1A, E2F, myc, and any other gene positively affecting the cell cycle regulatory system.

5. A method according to claim 4, wherein the gene coding for a cyclin is selected from the group consisting of a gene encoding a cyclin of B-type and a gene encoding a cyclin of D type.

6. A method according to any of claims 1-5, wherein the genetically modified plant is obtained by introducing into a cell of the parent plant a gene construct comprising a promoter and, operably linked thereto, a nucleotide sequence encoding a gene product that is involved in acceleration of growth in a tissue of the thus modified plant.

7. A method according to claim 6, wherein said gene product activates the cell cycle regulatory system of the plant.

8. A method according to claim 7, wherein the gene product is encoded by a geneselected from the group consisting of a gene coding for a cyclin, a gene coding for a transcription factor including E2F and myc, and any other gene positively affecting the cell cycle regulatory system.

9. A method according to claim 8, wherein the gene coding for cyclin is selected from the group consisting of the cyc1At gene (encoding a mitotic cyclin of B-type), the AtcycD2 gene (encoding a G1 cyclin of D-type) and the AtcycD1 gene (also encoding a G1 cyclin).

10. A method according to claim 6, wherein the promoter is a plant gene promoter.

11. A method according to claim 10, wherein the promoter is selected from the group consisting of an inducible promoter and a constitutive promoter.

12. A method according to claim 10, wherein the plant gene promoter is selected from the group consisting of an Atcdc2a promoter (prAtcdc2a), a 35S promoter and an Atcdc2b-promoter.

13. A method according to claim 6 wherein the gene construct comprises a poly-adenylation site.

14. A method according to claim 13, wherein the poly-adenylation site is derived from the Nopaline synthetase gene of *Agrobacterium tumefaciens*, an octopine synthetase gene or 35S polyadenylation sequences.

15. A method according to any of claims 6-14, wherein the gene construct is introduced by means of *Agrobacterium tumefaciens* or *Agrobacterium rhizogenez*.

16. A method according to any of the preceding claims, wherein the mutagenisation treatment of step (b) is performed by a method selected from the group consisting of EMS mutagenesis, T-DNA-mutagenesis and mutagenesis by using a transposable element.

17. A method according to any of the preceding claims, wherein the identification in step (d) of nucleic acid sequence(s) having a sequence which is different from the corresponding sequence(s) in the non-mutagenised transgenic plant is performed using a method selected from the group consisting of an Amplified Fragment Length Polymorphism (AFLP) method, a Single Sequence Length Polymorphism (SSLP), a differential display method, a restriction fragment length polymorphism (RFLP) method, a Single Strand Conformation Polymorphism (SSCP) method, allele specific amplification, restriction PCR, PCR, sequencing and a Single Nucleotide Polymorphism (SNP) method.

18. A method according to claim 17, wherein the identification method is an SSLP method.

19. A method according to any of the preceding claims, wherein the nucleic acid sequence identified in step (d) and/or the product encoded by the sequence is functionally associated with the phenotype of the selected mutant plants of step (c).

20. A method according to any of the preceding claims, wherein, in step (e), the target nucleic acid sequence is identified by a homology search in a genome database for the target organism or by molecular probing.

21. A method according to claims 20, wherein the molecular probing is carried out using a method selected from the group consisting of PCR, northern blotting, Southern blotting, arraying and direct sequencing.

22. A method according to any of the preceding claims comprising the further step of isolating the target nucleic acid sequence identified in step (e).

23. A method according to any of the preceding claims, wherein the product of the target nucleic acid sequence is functionally active in a signal transduction cascade leading to suppression of cell growth in the target organism.

24. A method according to any of the preceding claims, wherein the product of the target nucleic acid sequence is a suppressor of cell growth in the target organism.

25. A method according to any of the preceding claims, wherein a putative functional association between the plant nucleic acid sequence identified in step (d) and the target nucleic acid sequence is determined by homology analysis between said plant nucleic sequence and said target nucleic sequence.

26. A method according to any of the preceding claims, wherein a putative functional association between the plant nucleic acid sequence identified in step (d) and the target nucleic acid sequence is determined by analysing the effect of expressing the target nucleic acid sequence in an *in vitro* model for assaying cell growth regulation activity.

27. A method according to any of the preceding claims, wherein a putative functional association between the plant nucleic acid sequence identified in step (d) and the target nucleic acid sequence is determined by analysing the effect of expressing the target nucleic acid sequence in an *in vivo* model for assaying cell growth regulation activity.

28. A method according to any of the preceding claims, wherein the eukaryotic target organism is a cell selected from the group consisting of a microbial cell, a plant cell and a mammalian cell.

29. A method according to claim 28, wherein the microbial cell is a yeast cell.

30. A method according to claim 28, wherein the mammalian cell is a cell of a mammal selected from the group consisting of insects, birds, mice, rats, guinea pigs, cats, dogs, apes, primates including humans.

31. A method of determining the tumour suppressor activity, if any, of a gene product encoded by a eukaryotic cell gene, the method comprising the steps of:

(a) providing a wild type plant or a plant that is genetically modified to have tissue exhibiting, relative to the tissue of its non-genetically modified parent plant, accelerated growth,

(b) subjecting a multiplicity of the wild type plant or the genetically modified plant or parts thereof to a mutagenisation treatment,

(c) selecting from the thus treated plants or parts thereof a mutant plant having, relative to the wild type plant or the genetically modified plant of step (a), a phenotype characterised by an altered morphological structure or an altered colour,

(d) identifying in said selected mutant plant a nucleic acid sequence having a sequence which is different from the corresponding sequence in the non-mutagenised wild type plant or genetically modified plant, and, using said different nucleic acid, identifying in the eukaryotic cell a homologue or analogue gene putatively involved in cell cycle regulation,

(e) transforming the coding sequence of said homologue or analogue gene into a mutant plant of step (c) under conditions permitting the sequence to be expressed, and

(f) determining whether or not the thus transformed mutant plant reverts to its wild type phenotype, such reversion being indicative of tumour suppressor activity of the homologue or analogue gene product.